



LAWRENCE  
LIVERMORE  
NATIONAL  
LABORATORY

## **Technical Basis Document**

# **A Statistical Basis for Interpreting Urinary Excretion of Plutonium Based on Accelerator Mass Spectrometry (AMS) Data from the Marshall Islands**

K.T. Bogen  
T.F. Hamilton  
T. A. Brown  
R.E. Martinelli  
A.A. Marchetti  
S.R. Kehl  
R.G. Langston

**October 2006**

## **DISCLAIMER**

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

## **Technical Basis Document**

### **A Statistical Basis for Interpreting Urinary Excretion of Plutonium Based on Accelerator Mass Spectrometry (AMS) for Selected Atoll Populations in the Marshall Islands**

K.T. Bogen, T.F. Hamilton, T. A. Brown, R.E. Martinelli, A.A.  
Marchetti, S.R. Kehl, and R.G. Langston

Marshall Islands Program  
Lawrence Livermore National Laboratory

**October 2006**

## Table of Contents

Summary .....	1
Introduction .....	1
Methods and Procedures.....	2
Methods for Statistical Analysis of AMS Plutonium Bioassay Data.....	3
Results.....	5
Discussion .....	12
Conclusion .....	13
Acknowledgement .....	13
References .....	1

## List of Figures

Figure 1. Annual Northern hemisphere deposition of $^{137}\text{Cs}$ produced in atmospheric nuclear testing.....	4
Figure 2. Cumulative respiratory exposure of program participants in the Marshall Islands to worldwide fallout contamination from atmospheric nuclear testing plotted as a function of volunteer age at the time of sample collection.....	7
Figure 3. Empirical cumulative probability mass functions (cmfs) for deviations between measures of $^{239}\text{Pu}$ in urine collected only once from study participants (both islands) and their corresponding age-group specific mean values (both curve).....	8
Figure 4. Empirical cumulative probability mass functions (cmfs) for $^{239}\text{Pu}$ measured by AMS in field blanks prepared on Enewetak ( $n = 29$ ) and on Rongelap ( $n = 21$ ). .....	8
Figure 5. Empirical cumulative probability mass functions (cmfs) for $^{239}\text{Pu}$ measured by AMS in combined field blanks vs. that measured in urine samples collected from 19 total females and males on Enewetak (left plot), and from 115 males on Rongelap (right plot.).....	10
Figure 6. Urinary $^{239}\text{Pu}$ is significantly positively associated both with age (left plot), and with estimated relative exposure to $^{239}\text{Pu}$ from worldwide fallout, For all (male and female) Enewetak participants ( $n = 94$ ), as assessed by Weighted linear regression using inverse-square measurement errors as weight (solid horizontal lines = $-0 \mu\text{Bq}/24\text{-h}$ ). .....	10

## List of Tables

Table 1. Summary of $^{239}\text{Pu}$ bioassay samples from Enewetak and Rongelap participants. ....	2
Table 2. Comparison of $^{239}\text{Pu}$ levels in 24-h urine assessed by Monte Carlo compound Mann-Whitney test. ....	6
Table 3. Fraction of samples containing $>0.35\ \mu\text{Bq } ^{239}\text{Pu}$ versus volunteer age.....	11

## SUMMARY

We have developed refined statistical and modeling techniques to assess low-level uptake and urinary excretion of plutonium from different population group in the northern Marshall Islands. Urinary excretion rates of plutonium from the resident population on Enewetak Atoll and from resettlement workers living on Rongelap Atoll range from <1 to 8  $\mu$ Bq per day and are well below action levels established under the latest Department regulation 10 CFR 835 in the United States for *in vitro* bioassay monitoring of  $^{239}\text{Pu}$  (Hamilton et al., 2005). However, our statistical analyses show that urinary excretion of plutonium-239 ( $^{239}\text{Pu}$ ) from both cohort groups is significantly positively associated with volunteer age,

especially for the resident population living on Enewetak Atoll. Urinary excretion of  $^{239}\text{Pu}$  from the Enewetak cohort was also found to be positively associated with estimates of cumulative exposure to worldwide fallout. Consequently, the age-related trends in urinary excretion of plutonium from Marshallese populations can be described by either a long-term component from residual systemic burdens acquired from previous exposures to worldwide fallout or a prompt (and eventual long-term) component acquired from low-level systemic intakes of plutonium associated with resettlement of the northern Marshall Islands, or some combination of both.

## INTRODUCTION

Researchers from the Center for Accelerator Mass Spectrometry (CAMS) at the Lawrence Livermore National Laboratory (LLNL) have recently developed a new technology for low-level detection and measurement of heavy elements based on accelerator mass spectrometry (AMS) (Brown et al., 2004). The accelerator mass spectrometry (AMS) system has a detection sensitivity of < 1  $\mu$ Bq for plutonium-239 ( $^{239}\text{Pu}$ ) (Brown et al., 2004; Hamilton et al., 2004) and is currently being used for routine analysis of plutonium bioassay samples collected from potentially exposed populations in the northern Marshall Islands (Hamilton et al., 2005). Under an approved protocol, over 400 individual bioassay samples have been collected under the

Marshall Islands Urinalysis Program over the last five years and analyzed for plutonium isotopes using AMS. All sample analyses were performed on 24-h void urine samples collected under carefully controlled conditions and analyzed for plutonium-239 ( $^{239}\text{Pu}$ ) and plutonium-240 ( $^{240}\text{Pu}$ ) with accompanying field blanks.

This technical basis document describes statistical and modeling methods developed to aid interpretation of plutonium urinary excretion data based on high-quality AMS measurements, and the corresponding results obtained by applying these methods to data obtained under the Marshall Islands Urinalysis Program at LLNL. The study was undertaken

specifically to determine whether elevated levels of plutonium excretion could be observed in different cohort population groups, if such elevations exhibit any age- or gender-related patterns, and identify how such patterns may relate to (1) cumulative exposure to

historical worldwide fallout; (2) occupational exposure of workers engaged in cleanup activities or agricultural practices; and (3) long-term chronic exposure of resident atoll populations in the Marshall Islands.

## METHODS AND PROCEDURES

Bioassay samples were collected from two separate cohorts: (1) a relative homogenous group of Marshallese living on Enewetak Island (Enewetak Atoll), and (2) a heterogeneous group of adult male contract workers, of mostly Marshallese decent, engaged in cleanup and rehabilitation programs on Rongelap Island (Rongelap Atoll). Associated fields blanks were prepared

using identical methods except for the substitution of 18 Megohm deionized H<sub>2</sub>O for urine. An outline of the samples analyzed by data group is shown in Table 1. A full description of the sample collection and measurement techniques used for low-level bioassay measurements of plutonium isotopes by accelerator mass spectrometry will be given elsewhere (Hamilton et al., 2005).

**Table 1.** Summary of <sup>239</sup>Pu bioassay samples from Enewetak and Rongelap participants.

Atoll group	Source	Number of samples	Number of volunteers	Volunteer Age (y) <sup>a</sup>		
				Median	Minimum	Maximum
Enewetak	Field Blank <sup>a</sup>	29	N/A	N/A	N/A	N/A
	Female	83	73	31.8	13	67
	Male	157	124	38.2	13	88
Rongelap	Field Blank <sup>a</sup>	21	N/A	N/A	N/A	N/A
	Male	167	115	36.1	18	66

<sup>a</sup> Field Blanks were prepared using deionized water rather than urine, but otherwise prepared and treated identically to urine bioassay samples, Volunteer ages were rounded to nearest year.

## METHODS FOR STATISTICAL ANALYSIS OF AMS PLUTONIUM BIOASSAY DATA

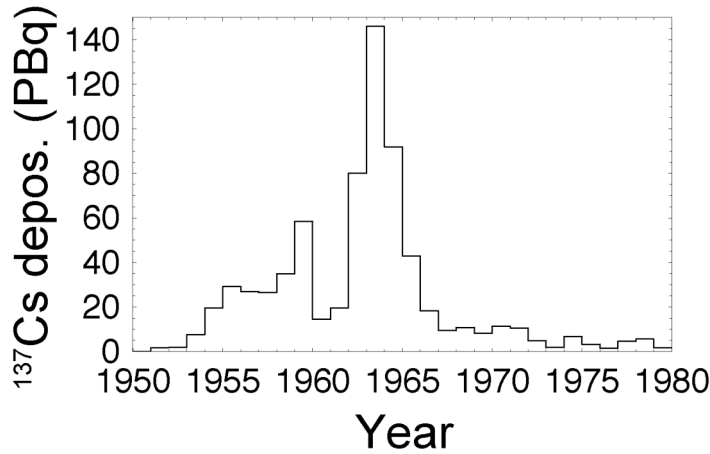
A total of 457 sample measurements (including field blanks) with associated uncertainties were used in the statistical analyses. The uncertainties of each measurement were propagated from the combined uncertainty of the individual bioassay measurement and those of the associated reagent blanks and AMS target background counts. For each set of plutonium measurement data described, traditional parametric and nonparametric tests for comparisons were not directly applicable because of the heterogeneous nature of individual measurement errors and relative errors. Moreover, multiple bioassay samples were collected from single participants from each population group. For each such individual, a corresponding weighted average value;  $\bar{x}_j = \frac{\sum_{i=1}^{n_j} w_i x_{i,j}}{\sum_{i=1}^{n_j} w_i}$  was calculated with  $w_i = s_{i,j}^{-2}$  for reported measurement errors  $s_{i,j}$ .

Volunteer participants on Enewetak and Rongelap Atolls were grouped by age into three age ranges corresponding to three divergent (low, medium and high) levels of estimated relative exposure to historical levels of fallout due to atmospheric nuclear testing. To this end, annual Northern hemisphere deposition of  $^{137}\text{Cs}$  produced in atmospheric nuclear testing (Figure 1; UNSCEAR, 2000) were used to estimate cumulative  $^{239}\text{Pu}$  exposure that might account in some part for any observed accumulation of elevated  $^{239}\text{Pu}$  levels in urine as a function of age, by integrating the area under the function plotted in Figure 1 from birth to the date of urine sampling for each

volunteer. Activity ratios of  $^{239+240}\text{Pu}$  to  $^{137}\text{Cs}$  measured at various locations in the Northern hemisphere during and subsequent to atmospheric testing are known to have remained fairly constant at about 1% (Perkins and Thomas, 1980). Although  $^{137}\text{Cs}$  fallout levels varied substantially by latitude during and beyond this period, the patterns over time at different latitudes were approximately proportional (UNSCEAR, 2000) and serve as a meaningful estimate of potential  $^{239}\text{Pu}$  exposure due to worldwide fallout. Inhalation would have accounted for ~95% of urinary  $^{239}\text{Pu}$  partitioning from blood concentrations that arise primarily from concentrations of  $^{239}\text{Pu}$  in stored bone, kidney and liver (Martin and Bloom, 1980).

Intra-individual variation among corresponding deviations  $d_{i,j} = x_{i,j} - \bar{x}_j$  was compared to inter-individual variation among combined age-group-specific deviations  $d_{h \neq i} = x_{h \neq i} - \bar{x}$  between remaining non-replicate measures  $x_{h \neq i}$  and each corresponding, age-group-specific weighted mean value  $\bar{x}$ , using a compound, semi-parametric version of the usual nonparametric Wilcoxon test for variance homogeneity. Whereas the standard Wilcoxon mean-homogeneity test assigns ranks to the ordered set of combined, ordered sample values from each of two sources (Lehman and D'Abrera, 1975), the test for unequal dispersion assigns a rank of 1 to the lowest of these sample values, but then assigns each adjacent pair of ranks in





**Figure 1.** Annual Northern hemisphere deposition of  $^{137}\text{Cs}$  produced in atmospheric nuclear testing (UNSCEAR, 2000). Cumulative  $^{239}\text{Pu}$  exposure that may partially explain observed accumulation of elevated  $^{239}\text{Pu}$  in urine as a function of age was estimated by (exact) integration of the area under the plotted function, from birth to the date of urine sampling for each participant.

alternating fashion to corresponding pairs of values that are first lowest, then highest, then next lowest, etc. The compound, semi-parametric version of the standard variance test was implemented by nested Monte-Carlo sampling of 100 sets each of  $d_{ij}$  and  $d_{h \neq i}$  from linearly smoothed versions of their respective empirical distribution functions, and each deviation  $d$  in each of the sample sets was itself sampled 10 times assuming  $d \sim N(d, s^2)$  in which  $s$  is the reported measurement error corresponding to  $d$ . The total of 1000 p-values assessing the likelihood of equal inter- versus intra-individual dispersion thus generated were sorted to evaluate the null hypothesis that  $X=Y$ .

Because less intra- than inter-individual variance was observed (see under Results), each set of  $n_j$  multiple measures  $x_{ij}$  obtained for a  $j$ th individual were replaced by its corresponding single weighted average value  $x_j$  defined above, and each corresponding set of

reported measurement errors  $s_{ij}$  was likewise replaced by a single estimate  $s_{wj}$  of the standard error of the weighted mean  $x_j$ , where:  $s_{wj}^2 = \text{Max}(s_x[n_j/(1 + \gamma_w^2)]^{1/2}, s_{\min})$ ,  $s_x$  is the sample variance of  $x_{ij}$ ,  $\gamma_w$  is the sample coefficient of variation (i.e., standard deviation divided by the mean) of weights  $w_i$ , and (to avoid spuriously low  $s_{wj}^2$  values)  $s_{\min} = \text{Min}(s_{ij})$  over all  $i$  and  $j$ .

The Mann-Whitney version of the Wilcoxon test referred to above was modified to compare one set of measures  $x_i$  ( $i = 1, \dots, n_x$ ) of  $X$  to another set  $y_j$  ( $j = 1, \dots, n_y$ ) of  $Y$ , conditional on each  $k^{\text{th}}$  measure  $z_k$  (for  $z = x$  or  $z = y$ ) being normally distributed as  $\sim N(z_k, s_{z,k}^2)$ , where  $s_{z,k}$  is the  $k^{\text{th}}$  corresponding reported measurement error. To implement this compound Mann-Whitney test by nested Monte Carlo sampling, a linearly smoothed version of the sample distribution function ( $F_x$ ) for  $x_i$ , and a similar function ( $F_y$ ) for  $y_j$ , were used to draw 100 sets of

$\{x_i^*, y_j^*\}$  sample vectors each of dimension  $\{n_x, n_y\}$ . Each sample vector set  $\{x_i^*, y_j^*\}$  was in turn used to draw 10 new sets  $\{x_{i,h}^{**}, y_{j,h}^{**}\}$ ,  $h = 1, \dots, 10$ , by sampling  $N(x_i^*, s_{x,i}^2)$  and  $N(y_j^*, s_{y,j}^2)$  ten times for each  $i$  and  $j$ . The unbiased estimate of  $P = \text{Prob}(X > Y)$  is the area under the corresponding receiver operating characteristic (ROC) curve (Zweig and Campbell, 1993). The distribution  $F_P$  of  $P$  was estimated from 1000  $P$  values each calculated by the usual exact ROC method (Lehman and D'Abrera, 1975) applied to a simulated set  $\{x_{i,h}^{**}, y_{j,h}^{**}\}$ . The estimate  $\hat{F}_P$  was in turn used to estimate (a) the expected  $P$ -value  $\hat{P}$ , (b) 2-tail lower and upper 95% confidence limits on  $P$ , and (c) a 2-tail significance value  $\text{Prob}(X=Y)$  of the null hypothesis that  $X=Y$ , with the latter quantity evaluated as  $p = \text{Min}[2 \text{ Min}[\text{Prob}(X \leq 0.50), \text{Prob}(Y \leq 0.50)], 1]$  as is the case for the usual Mann-Whitney or equivalent Wilcoxon rank sum test (Lehman and D'Abrera, 1975).

Sample age distributions were compared using the Kolmogorov 2-sample test (Wilcox, 1997). Sample distributions of measured  $^{239}\text{Pu}$  levels,

weighted by corresponding  $1/s_{z,k}^2$  values, were compared graphically to supplement the more rigorous Monte Carlo compound Mann-Whitney test described above. Association of  $^{239}\text{Pu}$  levels with age, and with relative fallout-Pu exposure) was assessed by weighted least-squares linear regression, using  $1/s_{z,k}^2$  as weights, reporting the corresponding squared correlation coefficient ( $R^2$ ) and 2-tailed significance level for non-zero slope. Regression slopes were compared using similarly weighted analysis of covariance (ANOCOVA). Additional tests for 2x2 association at each age range relative to field blank data, for overall homogeneity, and for overall trend in the fraction of elevated levels of  $^{239}\text{Pu}$ , were done heuristically (ignoring measurement errors) using Fisher exact tests, extended Fisher exact tests (Baglivo *et al.*, 1988), and Bartholemew's trend tests (Fleiss, 1981), respectively. All calculations were done using *Mathematica* 5.0<sup>®</sup> (Wolfram, 1999) and *RiskQ* (Bogen, 2002) software. All p-values  $< 10^{-10}$  are reported as being  $\sim 0$ .

## RESULTS

Comparisons of AMS  $^{239}\text{Pu}$  measures by data group (urine sample, field blank or positive control), by island, by gender and by age are summarized in Table 1, and corresponding comparisons done using the compound Mann-Whitney test are summarized in Table 2. Figure 2 shows estimated relative levels of cumulative historical

exposure to  $^{239}\text{Pu}$  fallout due to atmospheric nuclear tests, due primarily from inhalation of suspended  $^{239}\text{Pu}$ , plotted as a function of participant age. This plot reveals three age ranges that correspond to the following estimates of (unitless) relative fallout on each island (listed here  $\pm 1$  SDM):  $< 35$  y old ( $0.020 \pm 0.003$  for Enewetak,  $0.050 \pm 0.005$  for Rongelap), 35-44 y old ( $0.403 \pm 0.043$

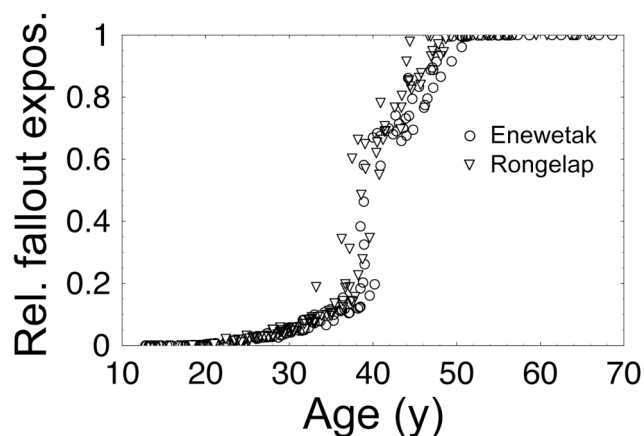
**Table 2.** Comparison of  $^{239}\text{Pu}$  levels in 24-h urine assessed by Monte Carlo compound Mann-Whitney test.

Group 1 (X) <sup>a</sup>	Wt. Mean of X ( $\mu\text{Bq}$ ) <sup>b</sup>	Standard Error of Wt. Mean ( $\mu\text{Bq}$ ) <sup>b</sup>	$n_1$	Group 2 (Y) <sup>a</sup>	$P = \text{Prob}(X>Y)^c$			$p = \text{Prob}(X=Y)^c$
					$\hat{P}$	95% LCL	95% UCL	
Enewetak FB	-0.027	0.067	29					
Rongelap FB	0.001	0.030	21	Enewetak FB	0.53	0.33	0.74	0.78
All FB	-0.016	0.040	50					
Enewetak <35 y	0.046	0.17	105	All FB	0.58	0.46	0.69	0.15
Enewetak 35-44 y	0.19	0.085	41	All FB	0.67	0.51	0.81	<b>0.037</b>
Enewetak $\geq 45$ y	0.30	0.32	51	All FB	0.70	0.57	0.82	<b>~0</b>
Enewetak M <35 y	0.062	0.21	73	All FB	0.59	0.47	0.72	0.15
Enewetak M 35-44 y	0.24	0.11	20	All FB	0.64	0.44	0.83	0.16
Enewetak M $\geq 45$ y	0.24	0.49	31	All FB	0.70	0.52	0.85	<b>0.030</b>
Enewetak F <35 y	0.025	0.28	32	All FB	0.56	0.41	0.72	0.42
Enewetak F 35-44 y	0.17	0.13	21	All FB	0.67	0.49	0.84	0.074
Enewetak F $\geq 45$ y	0.41	0.28	20	All FB	0.72	0.53	0.88	<b>0.048</b>
Rongelap <35 y	0.044	0.12	51	All FB	0.60	0.46	0.75	0.14
Rongelap 35-44 y	0.18	0.15	42	All FB	0.68	0.54	0.82	<b>0.012</b>
Rongelap $\geq 45$ y	0.10	0.52	22	All FB	0.70	0.50	0.88	0.054
All Control	0.16	0.19	22	All FB	0.71	0.53	0.87	<b>0.028</b>

<sup>a</sup> A name and (as applicable) age range (in y) are listed for each data group. M = male; F = Female; FB = field blanks (samples of double-distilled  $\text{H}_2\text{O}$  rather than urine, but otherwise prepared and treated identically to urine samples); Control = urine samples obtained from >5 Marshallese individuals (primarily from Rongelap and Enewetak).

<sup>b</sup> Wt. mean = weighted sample mean of  $n_i$  total measures (for  $i = 1$  or 2) using  $n_i$  corresponding values of  $\text{SE}^{-2}$  as sample weights, where SE = corresponding combined standard error of AMS sample preparation and measurement.

<sup>c</sup> Evaluated by Monte Carlo compound Mann-Whitney test.



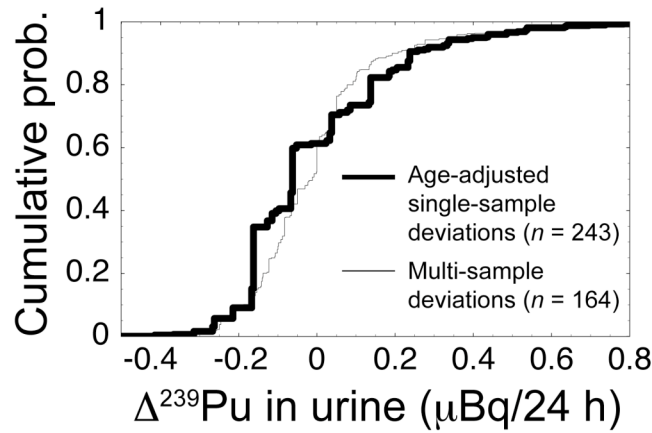
**Figure 2.** Cumulative respiratory exposure of program participants in the Marshall Islands to worldwide fallout contamination from atmospheric nuclear testing plotted as a function of volunteer age at the time of sample collection.

for Enewetak,  $0.478 \pm 0.045$  for Rongelap), and  $\geq 45$  y old ( $0.963 \pm 0.009$  for Enewetak,  $0.967 \pm 0.011$  for Rongelap), where 35-44 y denotes the 10-y interval at which age is  $\geq 35$  y and  $< 45$  y.

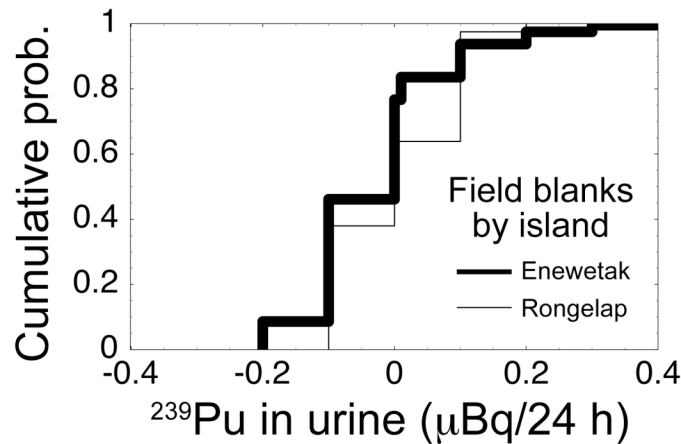
Figure 3 compares two cumulative probability mass functions (cmfs) involving differences between measured and corresponding mean  $^{239}\text{Pu}$  levels for study participants (both islands): one “inter-individual variability” cmf of differences for 243 participants who were sampled only once (subtracting age-group-specific mean values), and one “intra-individual variability” cmf of differences between 164 measures collected on  $\geq 2$  occasions from 69 other participants (subtracting corresponding participant-specific mean values). The 243 singly sampled and 69 multiply sampled participants had mean ( $\pm 1$  SEM) ages of  $34.6 \pm 0.9$  y and  $38.3 \pm$

$1.6$  y, respectively; the corresponding age distributions do not differ significantly ( $p = 0.14$ , by Kolmogorov 2-sample test). The two sets of deviations (that by definition. the same weighted-mean value of 0) do not differ significantly by 2-tail compound Mann-Whitney test ( $p = 1$ ). The variance of intra-individual deviations is  $\sim 57\%$  of that of the inter-individual deviations, but this could be due to chance as assessed by compound Wilcoxon-variance test ( $p = 0.96$ ).

$^{239}\text{Pu}$  levels measured in field blanks prepared on Enewetak and Rongelap are summarized in Figure 4. As sets of field-blank measures made on each island on do not differ significantly ( $p = 0.78$ , Table 2), all field blanks were pooled in subsequent statistical comparisons. Estimated likelihoods  $\hat{p}$  that  $^{239}\text{Pu}$  measures from participants’ urine samples are greater than those



**Figure 3.** Empirical cumulative probability mass functions (cmfs) for deviations between measures of  $^{239}\text{Pu}$  in urine collected only once from study participants (both islands) and their corresponding age-group-specific mean values (bold curve), and for such deviations between measures collected on  $\geq 2$  occasions from the remaining participants and their corresponding participant-specific mean values (light curve). Each cmf shown is weighted by the inverse squares of corresponding measurement errors associated with each contributing measure. The two sets of deviations have the same weighted-mean value of 0 and do not differ significantly by 2-tail compound Mann-Whitney test ( $p = 1$ ). The variance of intra-individual deviations is 57% of that of the inter-individual deviations.



**Figure 4.** Empirical cumulative probability mass functions (cmfs) for  $^{239}\text{Pu}$  measured by AMS in field blanks prepared on Enewetak ( $n = 29$ ) and on Rongelap ( $n = 21$ ). Each cmf is weighted by the inverse squares of corresponding measurement errors associated with each contributing measure. The two sets of measures do not differ significantly by 2-tail compound Mann-Whitney test (0.78).

from combined field blanks are listed in column 6 of Table 2. The sets of age-

specific likelihood estimates obtained for each island, and those obtained for

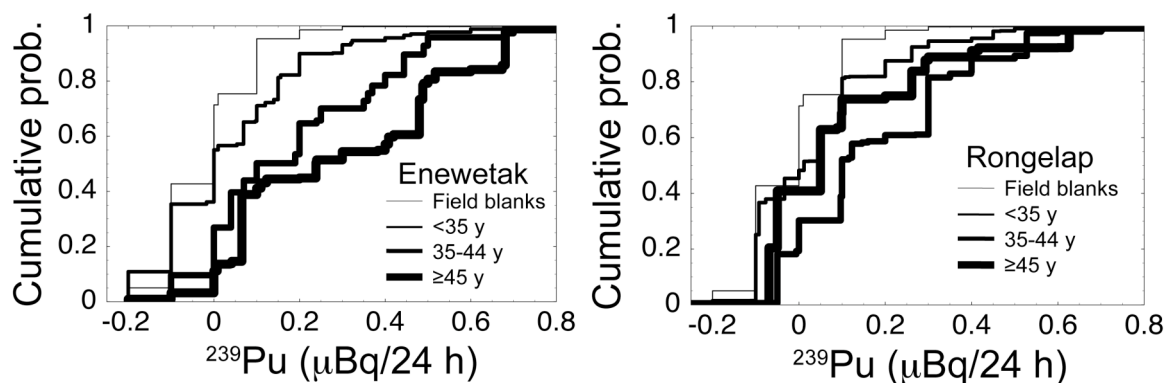
each sex on Enewetak, in each case increase by age across the three age groups considered.

The most significantly elevated  $^{239}\text{Pu}$  measures were observed in samples from older participants (of age  $\geq 45$  y) on Enewetak compared to combined field blanks ( $p = \sim 0$ ). However, significantly elevated levels were detected in samples from at least one age group within each of the four sets of island- and sex-specific data sets studied (see bold p-values listed in rightmost column of Table 2).

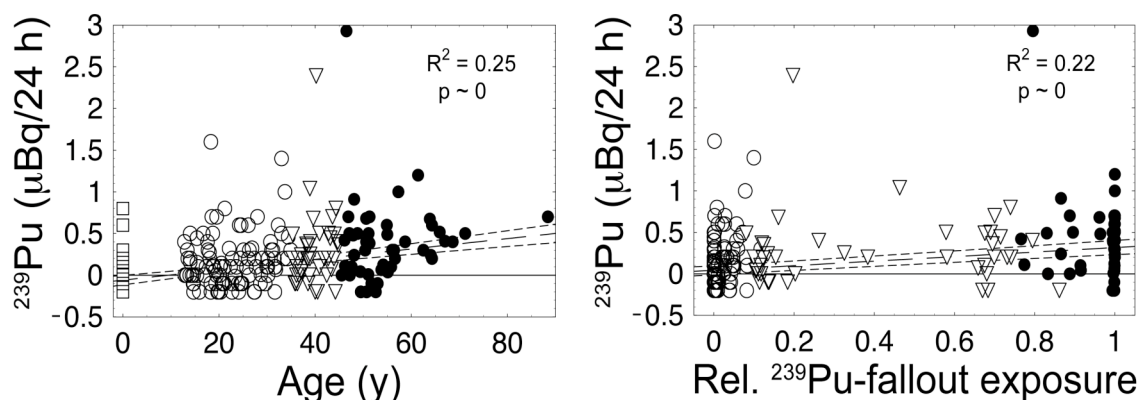
Figure 5 compares cmfs for  $^{239}\text{Pu}$  measured in all field blanks combined with those of urine samples collected within three age groups on each island. An age-related trend is more evident for Enewetak than for Rongelap, although based on the compound Wilcoxon test, the trend for increasing  $^{239}\text{Pu}$  is monotonically related to age on each island (Table 2). Using combined field blank data as a baseline reference group, pan-group non-homogeneity of  $^{239}\text{Pu}$  levels and a corresponding age-related trend on both islands is also

supported heuristically by Fisher exact, extended Fisher exact and Bartholomew's trend tests performed, without regard to measurement error, on the fractions of  $^{239}\text{Pu}$  values  $> 0.35 \mu\text{Bq}$  per 24-h in each age range (Table 3).

Even when  $^{239}\text{Pu}$  data for the youngest age group are used as the reference group (i.e., excluding field blank data from the trend analysis), a significantly positive linear trend with age is evident in the Enewetak data set (Table 3). Nearly all ( $> 98\%$ ) of 312 individual-specific urinary  $^{239}\text{Pu}$  levels derived from both islands were outside a range of  $-1$  to  $5 \mu\text{Bq}$ . Figure 6 plots urinary  $^{239}\text{Pu}$  levels in this range vs. age (left plot), and vs. relative exposure to fallout- $^{239}\text{Pu}$  (right plot), for all (male and female) Enewetak donors, together with corresponding weighted linear fits. Both plots show a highly significant positive trend ( $p \approx 0$ ). A significantly positive age-related trend in urinary  $^{239}\text{Pu}$  was also found in corresponding data for all (male) Rongelap donors ( $p = 0.00017$ ), but with a slope significantly less than that for Enewetak ( $p = 0.0036$ ), and no significant association between urinary  $^{239}\text{Pu}$  and relative fallout exposure was evident using Rongelap data ( $p = 0.092$ ).



**Figure 5.** Empirical cumulative probability mass functions (cmfs) for  $^{239}\text{Pu}$  measured by AMS in combined field blanks vs. that measured in urine samples collected from 194 total females and males on Enewetak (left plot), and from 115 males on Rongelap (right plot). Each cmf is weighted by the inverse squares of corresponding measurement errors associated with each contributing measure. Urinary  $^{239}\text{Pu}$  levels are significantly elevated among all participants 35 to 44 y old on each island ( $p < 0.05$ ), and among participants  $\geq 45$  y old on Enewetak ( $p \approx 0$ ), by 2-tail compound Mann-Whitney test.



**Figure 6.** Urinary  $^{239}\text{Pu}$  is significantly positively associated both with age (left plot), and with estimated relative exposure to  $^{239}\text{Pu}$  from worldwide fallout, for all (male and female) Enewetak participants ( $n = 194$ ), as assessed by weighted linear regression using inverse-square measurement errors as weights (solid horizontal lines =  $0 \mu\text{Bq}/24\text{-h}$ ). In each plot,  $\circ = <35$  y,  $\nabla = 35\text{-}44$  y,  $\bullet = \geq 45$  y; the left plot (and corresponding regression) includes pooled field blank ( $\square$ ) data ( $n = 50$ ). Both regressions exclude (3) measures  $< -1$  or  $> 5 \mu\text{Bq}$ . Each plot shows an estimated linear fit (long-dashed line), and 95% confidence limits on that fit (short-dashed lines).

**Table 3.** Fraction of samples containing  $>0.35 \mu\text{Bq } ^{239}\text{Pu}$  versus volunteer age.<sup>a</sup>

Atoll	Sample group <sup>a</sup>	N	Number of measures $>0.35 \mu\text{Bq}$	p-values <sup>b</sup>		
				Association	Homogeneity	Trend
Enewetak	FB	50	2			
	<35 y	103	20	0.014	$6.9 \times 10^{-8}$	$1.9 \times 10^{-8}$
	35-44 y	41	14	0.00036	[0.00022]	$[4.8 \times 10^{-5}]$
	$\geq 45$ y	50	26	$6.2 \times 10^{-8}$		
Rongelap	FB	50	2			
	<35 y	50	11	0.016	0.00050	0.00029
	35-44 y	51	15	0.00018	[0.29]	[0.14]
	$\geq 45$ y	42	6	0.011		

<sup>a</sup> FB = All field blanks,  $N$  = total number of Field Blank measures (pooled from both islands) or number of volunteer measures obtained from each island for each age group listed. Measurement errors were ignored in these analyses, and only measured values between -1 and  $5 \mu\text{Bq}$  were included (measures for 3 Enewetak and 2 measures from the Rongelap group, respectively). Multiple samples from any single individual were pooled as explained above.

<sup>b</sup> Association with age (relative to Field Blank data) was assessed by 2-tail Fisher exact test; homogeneity and linear trend were assessed using extended Fisher exact tests for independence, and by Bartholemew's test for trend, respectively, in the implied  $2 \times k$ -fold contingency table with  $k=4$  ordered as listed by age group; p-values in brackets correspond to  $k=3$  with Field Blank data excluded.



## DISCUSSION

Under the auspices of the Office of International Health Studies, Office of Health Safety, U.S. Department of Energy, researchers from the Center for Mass Spectrometry at the Lawrence Livermore National Laboratory are providing radiological surveillance monitoring of island population groups in the northern Marshall Islands. The AMS detection system used to assess plutonium uptake and dose far exceeds the standard requirements established under the latest Department regulation 10 CFR 835 used in the United States for *in vitro* bioassay monitoring of  $^{239}\text{Pu}$  (Hamilton et al., 2004). This newly established bioassay program based on accelerator mass spectrometry clearly provides a more accurate and reliable basis to assess incremental intakes of plutonium in association with future resettlement programs in the northern Marshall Islands or from changes in existing dietary and/or land-use patterns (Hamilton et al., 2005).

Under present living conditions, the plutonium bioassay program established for the resident population on Enewetak Island and for resettlement workers living on Rongelap Island clearly show that the systemic uptake of plutonium from potential exposures to elevated levels of fallout contamination in the local environment is extremely low. Moreover, the amount of plutonium measured in bioassay samples collected from these groups is comparable to or less than that measured in a small number of non-Marshallese U.S. workers with no known previous exposure to plutonium besides that in

worldwide fallout (Bogen et al., 2004a-b). Interpretation of the bioassay data is complicated by the large uncertainties in individual measurements – most of which fall into a sub  $\mu\text{Bq}$  range or below the critical level of detection (Hamilton et al., 2005). Withstanding this, our detailed statistical analysis of the combined bioassay data using the methods outlined in this report and our high-quality AMS measurement data do show some interesting trends in that urinary excretion of plutonium is consistently low yet significantly positively associated with age. The significant age-related trend observed for Enewetak residents compares with a less pronounced (albeit still statistically significant) age-related trend observed for the Rongelap resettlement workers. Rongelap resettlement workers involved in soil remediation were expected to have a higher probability of receiving a significant increment of intake detectable above any baseline excretion from systemic burdens acquired from previous exposures (Hamilton et al., 2005). If this were true then we would not expect to see a significant age related trend in urinary excretion of plutonium in this cohort. The difference in the strength of the age-related trends between the two population groups is more likely due to the inter-cohort variability in volunteer age and island-specific residence time. For example, one of the selection criteria for the Enewetak cohort group was that all volunteers should have lived on Enewetak Island since the atoll was resettled in 1980 (Hamilton et al., 2005).

By comparison, resettlement workers on Rongelap lived on island for more limited and variable amounts of time

(i.e., from weeks to several years) interspersed with residence in other parts of the Marshall Islands.

## CONCLUSION

Advances in the quality and reliability of *in vitro* bioassay monitoring of  $^{239}\text{Pu}$  based on AMS detection and measurement appear to be provide new insights into the uptake and urinary excretion of plutonium in cohort populations from the Marshall Islands. Select island population groups monitored through 2005 show low-level age-related patterns consistent with either (1) a long-term urinary excretion component associated with systemic burdens acquired from cumulative exposure to worldwide fallout contamination or (2) a prompt (and eventual long-term) component acquired from low-level systemic intakes of plutonium associated with resettlement of the northern Marshall Islands. If hypothesis (1) is true, this study would be the first ever to document a human  $^{239}\text{Pu}$ -specific biomarker signature of worldwide  $^{239}\text{Pu}$  fallout. We would further predict that individuals born after about 1970 who now or may someday reside on the islands will continue to have and excrete background levels of plutonium that do not increase with age

(i.e., with duration of island residence). The alternative hypothesis (2) predicts that the age-related pattern of urinary plutonium excretion may be derived from chronic or small incremental intakes associated with resettlement of the northern Marshall Islands where residual levels of plutonium contamination in the environment are elevated over that expected in integrated worldwide fallout deposition. The strength of age-related trends in plutonium excretion across the two study cohort populations appears to be more consistent with hypothesis (2) because it would be reasonable to assume that both population groups have been similarly exposed to cumulative worldwide fallout but experience different intakes from local sources. In future work, we will assess baseline urinary excretion of plutonium from other population groups in the northern Marshall Islands and conduct a re-evaluation of exposure pathways, especially in relation to dietary intakes from consumption of fish and other marine products.

## ACKNOWLEDGMENTS

This work was performed under auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48, with funding from the U.S. DOE Office of International Health Studies.

## REFERENCES

- Baglivo, J, D Oliver, and M. Pagano (1988). Methods for the analysis of contingency tables with large and small cell counts, *J. Am. Statist. Assoc.*, **83**, 1006-1013.
- Bogen, K.T. (2002). *RiskQ 4.2: An Interactive Approach to Probability, Uncertainty and Statistics for use with Mathematica®*. UCRL-MA-110232 Rev 3. Lawrence Livermore National Laboratory, Livermore, CA.
- Bogen, K.T., A.A. Marchetti, and T.A. Brown (2004a). Use of a correlated compound-binomial model to assess absence of non-counting noise in Pu-isotope ratios measured by AMS at LLNL, *Nuclear Instr. Meth. Physics Res. B*, **223**, 209-215.
- Bogen, K.T., D.P. Hickman, T.F. Hamilton, T.A. Brown, C.C. Cox, A.A. Marchetti, and R.E. Martinelli (2004b). Application of Accelerator Mass Spectrometry to Analyze  $^{239}\text{Pu}$  in Archived Occupational Samples. LDRD 01-ERD-108 Final Report, UCRL-TR-20574, Lawrence Livermore National Laboratory, Livermore, CA.
- Brown T.A., A.A. Marchetti, R.E. Martinelli, C.C. Cox, J.P. Knezovich and T.F. Hamilton (2004). Actinide measurements by accelerator mass spectrometry at Lawrence Livermore National Laboratory, *Nucl. Instrum. Meth. Phys. Res. B*, **223**, 788-795.
- Fleiss, J.L. (1981). Statistical Methods for Rates and Proportions. 2nd ed. John Wiley & Sons, New York, NY, pp. 138-159.
- Hamilton T.F., T.A. Brown, D.P. Hickman, A.A. Marchetti, R.E. Martinelli, and S.R. Kehl (2004). *Low-Level Plutonium Bioassay Measurements at the Lawrence Livermore National Laboratory*, October 6, 2004; Lawrence Livermore National Laboratory, Livermore, CA; UCRL-MI-207082.
- Hamilton, T.F., T.A. Brown, A.A. Marchetti, R.E. Martinelli, S.R. Kehl, K.T. Bogen, and R.G. Langston (2005). *Low-level detection of plutonium isotopes in bioassay samples collected from Marshall Islands using accelerator mass spectrometry*, Lawrence Livermore National Laboratory, CA (in preparation).
- Lehman, E.L., and H.J.M. D'Abrera (1975). *Nonparametrics: Statistical Methods Based on Ranks*, Holden-Day Inc., San Francisco, CA.
- Martin, W.E., and S.G. Bloom (1980). *Nevada Applied Ecology Group Model for estimating plutonium transport and dose to man*. In: Hanson, W.C., ed. *Transuranic Elements in the Environment: A Summary of Environmental Research on Transuranium Radionuclides Funded by the U.S. Department of Energy Through Calendar Year 1979*, DOE/TIC-22800, U.S. Department of Energy, Washington, DC, pp. 459-512.

Perkins, R.W., and C.W. Thomas (1980). *Worldwide fallout*. In: Hanson, W.C., ed. *Transuranic Elements in the Environment: A Summary of Environmental Research on Transuranium Radionuclides Funded by the U.S. Department of Energy Through Calendar Year 1979*, DOE/TIC-22800, U.S. Department of Energy, Washington, DC, pp. 53-82.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2000). *UNSCEAR 2000 Report to the General Assembly, with Scientific Annexes. Volume 1: Sources*. United Nations, New York, NY. Annex C: (*Exposures to the public from man-made sources of radiation*), Figure VII, and Table 10 (Annual deposition of radionuclides produced in atmospheric nuclear testing, Northern hemisphere), pp. 167, 214-215.

Wilcox, R.R. (1997). Some practical reasons for reconsidering the Kolmogorov-Smirnov test, *Br. J. Math. Stat. Psychol.* **50**, 9-20.

Wolfram, S. (1999). *The Mathematica Book*. 4th ed. Cambridge University Press, Cambridge, UK.

Zweig, M.H., and G. Campbell (1993). Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine, *Clin. Chem.* **39**, 5.